REMARKS

Claims 17-18 as amended are pending in the application along with new claims 19-30 as added by this amendment. Claims 1-4 are canceled by this amendment; claims 5-16 were canceled at the time of filing.

For the Examiner's convenience, attached Exhibit 1 contains all pending claims as they would appear after amendment.

Claims 19-21 are added to point out more clearly various particular embodiments of applicants' invention. Support for claims 19-21 is found in the specification at page 4, lines 12-16 and page 17, lines 3-5. The language in claim 20 describing the "agent" parallels the language of claim 1 of U.S. Patent No. 5,206,345 (the application for which was the parent of the present divisional application).

Claims 22-29 are added to clarify that aspect of applicants' invention which involves the blocking of interaction between VCAM-1-expressing cells and VCAM-1-binding cells (see specification at page 4, lines 11-15). Support for claims 22-29 is found in the specification at page 14, line 28 to page 15, line 34, and page 17, lines 3-5 and 24-33. New claim 30 addresses applicants' invention in the context of applicants' novel discovery that bone marrow stromal cells express VCAM-1 (see specification at page 17, lines 3-5) and that bone marrow cells, especially those bearing the CD34 antigen, express VLA-4, a major receptor for VCAM-1 (see specification at page 17, lines 24-33).

The specification and abstract are amended to recite the American Type Culture Collection (ATCC) accession number of the hybridoma cell line that produces monoclonal antibody 6G10. A copy of the Budapest Treaty receipt and declaration for this cell line is submitted herewith.

The Claimed Subject Matter

Claims 17-30 relate to uses of agents that bind to vascular cell adhesion molecule-1 (VCAM-1). VCAM-1 is expressed on a number of connective tissue-type cells, including

endothelial and bone marrow stromal cells, and mediates interaction of the connective tissue-type cells with blood cells such as lymphocytes and bone marrow cells. VCAM-1 expression by connective tissue may be up-regulated by treatment with IL-4, IL-1β and other cytokines.

Applicants have discovered agents that specifically bind to VCAM-1 and possess the ability to block VCAM-1-mediated cell-cell interactions. A representative embodiment of these agents is monoclonal antibody 6G10 produced by hybridoma ATCC No. HB 10519.

Claims 17-19 are directed to a method of modulating the immune response by administering an agent that specifically binds to IL4-activated microvascular endothelial cells and impedes transmigration of cells that bind to VCAM-1. Claims 20-21 are drawn to the method of claim 17 wherein the agent binds to an epitope recognized by monoclonal antibody 6G10. Claims 22-29 are directed to methods of modulating interaction between a VCAM-1-expressing cell and a cell that binds to VCAM-1 by administering an agent that binds to an epitope recognized by monoclonal antibody 6G10. Claim 30 is drawn to a method of modulating interaction between a bone marrow stromal cell and a bone marrow cell by administering an agent that specifically binds to VCAM-1.

The Outstanding Rejections

Claims 1-4 and 17-18 were rejected under 35 U.S.C. § 112, first paragraph. The Examiner stated that the specification failed to provide a written description of the invention because it was missing an ATCC number. The Examiner also asserted that the specification lacked enablement for *in vivo* utility because it did not teach how to administer the active agents and disclosed only *in vitro* results.

Claims 2, 4, and 17-18 were rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicants regard as the invention. It was the Examiner's position that it was unclear whether claims 2 and 4 were drawn to the administration of IL-1β alone, or in combination with IL-4. It was also the Examiner's position that in claims 17-18 the term "an agent that

specifically binds to IL4-activated microvascular endothelial cells" did not clearly define the bounds of the claims.

Claims 1-4 were rejected under 35 U.S.C. § 103 as obvious over Thornhill et al. (ref. O52) or Masinovsky et al. (ref. R) in view of Munro et al. or Cotran (ref. O10). Claims 17-18 were rejected under 35 U.S.C. § 103 as obvious over Thornhill et al. and further in view of Osborn et al. (ref. O29).

A. The Rejection Under 35 U.S.C. § 112, First Paragraph, May Properly Be Withdrawn

The Examiner objected to the specification under 35 U.S.C. § 112, first paragraph, stating that it failed to provide a written description of the invention:

> It appears that "the agent that specifically binds to IL-4 activated microvascular endothelial cells" and the "binding partners that bind to the IL-4 activated microvascular endothelial cells" are the same "mAb6G10 produced by hybridoma with ATCC No." But it is noted on page 4, line 16, page 21, line 11, and page 29, line 18 of the specification that ATCC No. is missing.
>
> Therefore, applicant is advised to provide the missing information in response to this office action in order to overcome the objection to the specification.

In response, applicants have amended the specification to provide the information requested by the Examiner.

The Examiner also objected to the specification under 35 U.S.C. § 112, first paragraph, because it allegedly failed to provide enabling disclosure for the *in vivo* utility. The Examiner asserted that:

> In addition to the above, the specification lacks enablement for *in vivo* use of IL-4 or IL-1β as set forth in claims 1-4 and also *in vivo* use of "an agent that specifically binds to IL4-activated microvascular endothelial cells" as set forth in claims 17-18.
>
> The specification neither teaches how to administer the active agents nor what "an effective amount" for *in vivo* administration is.
>
> The disclosure of "inconclusive" result of IL-4 effect on lymphocyte adhesion suggesting that "VCAM-1 might contribute to the process of lymphocyte attachment" (see page 15, lines 11-14) to microvascular endothelial cells or the conclusion based on the *in vitro* experimental data, such as antibody binding assay (see page 15, lines 31-34) cannot be

> accepted as the evidence of *in vivo* activity of IL-4, because it is unpredictable as to how IL-4 would interact with other cytokines in *in vivo* settings and how the binding agent would react with other existing VCAMs in endothelial cells *in vivo*.

The Examiner rejected claims 1-4 and 17-18 under 35 U.S.C. § 112, first paragraph, for the same reasons as the objection to the specification.

The rejection of claims 1-4 is moot because those claims are canceled by this amendment. With respect to claims 17-21, applicants respectfully submit that the disclosure in the specification enables modulation of the immune response *in vivo* by administering an agent that specifically binds to IL4-activated microvascular endothelial cells. The specification also enables the methods of claims 22-30 for modulating interaction between a cell expressing VCAM-1 and a cell binding VCAM-1.

The specification need not disclose exact dosages for the claimed methods because one of ordinary skill in the art would know how to administer these substances and how to determine effective amounts of these substances. For example, in Vedder et al., "A Monoclonal Antibody to the Adherence-promoting Leukocyte Glycoprotein, CD18, Reduces Organ Injury and Improves Survival from Hemorrhagic Shock and Resuscitation in Rabbits", *J. Clin. Invest.* 81:939-944 (1988) (attached hereto as Exhibit 2), an anti-CD18 monoclonal antibody that inhibited neutrophil adherence *in vitro* was administered to rabbits at effective *in vivo* dosages that resulted in improved survival rate after hemorrhagic shock and resuscitation. See *Ex parte Skuballa*, 12 USPQ2d 1570, 1571 (Bd. Pat. App. Int. 1989), in which the Board stated (with regard to a claim reciting numerous diverse utilities) that:

> the skilled worker in this art could readily optimize effective dosages and administration regimens for each of the recited utilities. As is well known, the specific dosage for a given patient under specific conditions and for a specific disease will routinely vary, but determination of the optimum amount in each case can readily be accomplished by simple routine procedures.

The specification teaches how to obtain an agent that specifically binds to IL4-activated microvascular endothelial cells ("ECs") and discloses that an agent that specifically binds to IL4-activated microvascular ECs blocks up to 80% of lymphocyte adhesion to cultured

ECs (see Example 4 at page 15). Data from lymphocyte adhesion assays are predictive of an effect on immune response because "leukocyte adhesion to endothelium [is] a phenomenon that is a critical component of inflammation, immune injury, and, as has been recently learned, atherosclerosis." Cotran, "New Roles for the Endothelium in Inflammation and Immunity", *Am J. Pathol.*, 129:407-413 (1987) (ref. O10) (hereinafter "Cotran"), at page 409. Lymphocyte adhesion to vascular endothelium is the first step in the migration of lymphocytes from blood into tissues. Thornhill et al., "IL-4 Increases Human Endothelial Cell Adhesiveness for T cells but not for Neutrophils", *J. Immunol.*, 144:3060-3065 (1990) (hereinafter "Thornhill et al.") at page 3060.

In a previous *in vivo* study in baboons, the results of *in vitro* studies of antibody binding using monoclonal antibodies specific for various cell adhesion molecules were found to correlate with *in vivo* results. Munro et al., "Tumor Necrosis Factor and Interferon-γ Induce Distinct Patterns of Endothelial Activation and Associated Leukocyte Accumulation in Skin of *Papio Anubis*", *Am. J. Pathol.*, 135:121-133 (1989) (hereinafter "Munro et al."), at page 127.

Others in the art have noted that *in vitro* clinical data correlate to *in vivo* data. According to Cotran at page 411, ". . . clinical studies strongly suggest that endothelial activation occurs regularly in settings of inflammatory, immune, and neoplastic reactions associated with activated lymphocytes and monocytes and is similar in terms of its profile of expression of endothelial antigens to activation *in vitro*." See also Munro et al., at pages 121 and 127.

Thus, data from *in vitro* assays of lymphocyte adhesion to microvascular ECs are predictive of an *in vivo* effect on the immune response. Applicants' data showing *in vitro* inhibition of lymphocyte adhesion by an agent that specifically binds to IL4-activated microvascular ECs is sufficient evidence that the agent will impede *in vivo* lymphocyte transmigration as well, as claimed in claims 17-21. In addition, applicants' data showing that an agent that binds to a monoclonal antibody 6G10-recognized epitope inhibits adhesion between

VCAM-1-expressing cells (such as ECs) and cells that bind VCAM-1 (such as lymphocytes), demonstrates that the agent will modulate cell-cell interactions *in vivo* according to claims 19-30.

It is incumbent on the Examiner to first establish a *prima facie* case of nonenablement. *Ex parte Hitzeman*, 9 USPQ2d 1821, 1822 (Bd. Pat. App. Int. 1987). The Examiner has provided no reason to doubt the applicability of the *in vitro* results disclosed in the specification to *in vivo* situations.

The Examiner suggests that the binding agent of claims 17-18 would react with other existing VCAMs on ECs *in vivo*. However, there is no evidence of other VCAMs, and the Examiner does not explain how such a cross-reaction would prevent the binding agent from being effective as claimed. The Examiner has provided no other reasons to doubt the *in vivo* utility of claims 17-30.

Hence, because applicants have shown *in vivo* utility for the methods of claims 17-30, and have enabled one skilled in the art to use these methods, the rejection under 35 U.S.C. § 112, first paragraph, may properly be withdrawn.

B. The Rejection Under 35 U.S.C. § 112, Second Paragraph, May Properly Be Withdrawn

The Examiner rejected claims 2, 4, and 17-18 under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicants regard as the invention. The Examiner's rejection of claims 2 and 4 is moot because those claims are canceled by this amendment.

The Examiner asserted with respect to claims 17-18 that:

> Even though the functional limitation such as "an agent specifically binds to IL4-activated microvascular endothelial cells" is set forth, because the term "an agent" in claims 17-18 are not clearly defined, it would take undue experimentation to determine which agent would have the activity required to carry out the invention. Therefore, "agent" in the claims fails to set the metes and bounds of the claims.

Applicants respectfully disagree with the Examiner's position. The term "agent that specifically binds to IL4-activated microvascular endothelial cells" is not indefinite, because

the specification describes such agents and sets forth a detailed method (page 3, line 36 to page 4, line 16, and page 20, line 29 to page 21, line 12) for obtaining candidates for such agents and determining whether the candidates have the activity required to carry out the invention. In addition, the specification discloses a working example, monoclonal antibody 6G10, of an agent that specifically binds to IL4-activated microvascular endothelial cells.

The method disclosed in the specification for obtaining such an agent does not require undue experimentation. "[A] considerable amount of experimentation is permissible, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed. . ." *In re Wands*, 858 F.2d 731, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988) (quoting *In re Jackson*, 217 USPQ 804, 807). In *Wands*, screening of hybridomas for high-affinity monoclonal antibodies was determined to be routine.

Under *Wands*, factors to be considered in determining undue experimentation include (1) the quantity of experimentation, (2) the amount of direction or guidance presented, (3) the presence of working examples, (4) the nature of the invention, (5) the state of the prior art, (6) the relative skill of those in the art, (7) the predictability of the art, and (8) the breadth of the claims. In this case, the production of monoclonal antibodies is predictable and well known in the art, and those in the art are relatively highly skilled in these procedures. The quantity of experimentation is routine, and applicants have provided clear guidance in their specification, including a working example and a specific embodiment, monoclonal antibody 6G10.

Thus, because applicants' specification clearly defines "agent that specifically binds to IL4-activated microvascular endothelial cells," the rejection under 35 U.S.C. § 112, second paragraph, may properly be withdrawn.

C. The Rejection under 35 U.S.C. § 103 May Properly Be Withdrawn

The Examiner rejected claims 1-4 under 35 U.S.C. § 103 as being unpatentable over any one of Thornhill et al. (ref. 52) or Masinovsky et al. (Ref. R) in view of Munro et al. or Cotran (Ref. 10). The Examiner's rejection of claims 1-4 is moot because those claims are canceled by this amendment.

The Examiner also rejected claims 17-18 under 35 U.S.C. § 103 as being unpatentable over Thornhill et al. as applied to claims 1-4 above, and further in view of Osborn et al. (Ref. 29). The Examiner asserted that:

> Thornhill et al. reference is discussed above. Thornhill et al., however, do not disclose a specific vascular adhesion molecule involved in lymphocyte adhesion.
>
> Osborn et al. (Ref. 29) disclose a vascular cell adhesion molecule, VCAM-1, which is induced by cytokines on human endothelial cells and binds lymphocytes and suggests the involvement of VCAM-1 in the involvement of lymphocyte recruitment into inflammatory sites. (see page 1203).
>
> As discussed above, since it is well established in the art that the transmigration of lymphocytes across postcapillary venules requires the adhesion of lymphocytes to microvascular endothelial cells and the binding of specific lymphocytes occurs via a specific vascular cell adhesion molecule, one of ordinary skill in the art would also be motivated to use an agent that binds to cytokine-activated microvascular endothelial cells to block lymphocyte-EC adhesion, with a reasonable expectation of success in impeding transmigration of lymphocytes.

Applicants respectfully disagree with the Examiner's position that claims 17-18 are rendered obvious by Thornhill et al. or Osborn et al., "Direct Expression Cloning of Vascular Cell Adhesion Molecule 1, a Cytokine-Induced Endothelial Protein that Binds to Lymphocytes", *Cell* 59:1203-1211 (1989) (hereinafter "Osborn et al.").

A *prima facie* case of obviousness requires that (1) the prior art suggest to those of ordinary skill in the art that they should carry out the claimed process, and that (2) the prior art reveal a reasonable expectation of success if the claimed process is carried out. *In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438, 1442 (Fed. Cir. 1991). Both the suggestion and the expectation of success must be found in the references relied upon. *Id.*

Claims 17-18 are directed to a therapeutic method of modulating the immune response in a patient, which comprises administering to the patient an agent that specifically binds to IL4-activated microvascular endothelial cells, in an amount effective to impede transmigration of cells that specifically bind to VCAM-1 from blood across postcapillary venules into extracellular fluid in the patient.

Thornhill et al. disclose that IL-4 induces a selective increase in adhesiveness of ECs for T-cells but not for neutrophils, by a mechanism that does not involve LFA-1β (CD18) or ICAM-1 (CD54). Thornhill et al. do not identify the adhesion molecule involved, nor do they provide an agent capable of specifically binding to the adhesion molecule involved. Osborn et al. identify a cDNA coding for the cellular adhesion molecule VCAM-1. Munro et al. and Cotran teach that cytokines activate the endothelium to promote lymphocyte adherence and migration into the inflammatory site.

None of the cited references discloses or suggests the methods of claims 17-18. None of the references discloses or suggests an agent that specifically binds to IL4-activated microvascular ECs and impedes lymphocyte adhesion, nor do they disclose or suggest use of such agents for modulating immune response. None of the references even suggests that it would be desirable to obtain such an agent, or how to identify such an agent.

Without knowledge of a method for identifying an agent that specifically binds to IL4-activated microvascular ECs, and without knowledge of applicants' data showing that such an agent effectively inhibits lymphocyte adhesion to ECs, one of ordinary skill in the art cannot have a reasonable expectation of success if the methods of claims 17-18 are carried out.

With regard to new claims 19-30, applicants note that none of the references discloses the method of claims 19-30 for modulating interaction between VCAM-1-expressing cells and cells that bind to VCAM-1. None of the cited references discloses or suggests agents that bind to a mAb 6G10-recognized epitope, nor do they disclose or suggest a method of use for such agents. Similarly, none of the references discloses or suggests the particular monoclonal antibody 6G10 or methods for using this antibody.

Therefore, because the references cited by the Examiner do not suggest to one of ordinary skill in the art that the methods of claims 17-30 be carried out, and because the references do not provide a reasonable expectation of success in carrying out these methods, the Examiner has not established a *prima facie* case of obviousness, and the § 103 rejection may properly be withdrawn.

CONCLUSION

In light of the foregoing amendments and remarks, it is believed that claims 1-4 and 17-18 are now in condition for allowance, and an early notice thereof is solicited.

Respectfully submitted,

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January 25, 1994